"MINING EVIDENCES OF MOLECULAR ARMS RACE BETWEEN PRIMATE IMMUNE SYSTEM AND CERTAIN PATHOGENIC BACTERIAL STRAINS"

SYNOPSIS

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Background:

Interaction between species or groups of organisms; both cooperative and antagonistic is preeminent. This includes struggle between predators and prey, competition for mates, as well as between pathogenic microbes and their hosts (Haldane;1949).

Hosts and their pathogens are engaged in a never-ending struggle with the hosts evolving to escape pathogen infection and pathogens evolving to escape host defences. This is referred to as the arms race. The term has been borrowed into biology from the Cold War struggle between the US and USSR where every move by the US was countered by a similar move by the USSR and vice versa. The continuous arms race between host and their pathogens may also be explained through the red queen hypothesis which was initially proposed by Leigh Van Valen in 1973 while trying to explain survival as evasion of extinction through continuous evolution. He drew the tangent from the famous book of Sir Lewis Carrol 'Through the Looking Glass' where the Red Queen tells Alice 'It takes all the running you can do to keep in the same place'. In evolutionary terms, it means an organism must continuously adapt and evolve not just for reproductive advantage but also for survival in face of the challenges thrown by competing organisms. The Red Queen hypothesis also holds true for evolutionary arms race wherein the host and pathogen are continually evolving (running) to retain their status-quo.

The spatial distribution of the host and pathogen phenotypes (resistance/ability to infect) have provided valuable information on local adaptation or maladaptation (Kaltz *et al*; 1999, Burdon and Thrall; 2000, Laine; 2011, Sicard *et al;* 2007), and thereby on coevolutionary processes.

The coevolutionary process may be explained through two models namely the 'arms race' model and the trench warfare' model. While the 'arms race', model posits the

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occurrence of recurrent selective sweeps each favouring novel resistance and virulence alleles, the 'trench warfare', model favours balancing selection maintaining stable and longstanding polymorphism at loci involved in host–pathogen recognition (Bergelson *et al*; 2001, Chisholm *et al;* 2006, Tellier and Brown; 2007). Under the 'trench warfare' model, the same alleles are maintained over long time scales while in the 'arms race' model novel alleles are recurrently driven to fixation.

Recently, studies have begun to emerge revealing molecular details of conflict between various components of the host immune and pathogens including bacteria, virus, fungi, and other parasites (Mitchell *et al;* 2012; Jacobs *et al;* 2014). Consistent with the existence of conflict between hosts and their pathogens, immune system components have been shown to be among the most rapidly evolving genes in animal genomes (Sackton *et al*; 2007; Enard *et al*; 2016; Hawkins *et al*; 2019). The pathogen genome which is usually small have been known to rise to the occasion, through varied strategies for evading the host immune system (Haldane; 1949).

Therefore, the host-pathogen evolution is a wonderful system which can provide invaluable glimpses into the evolutionary battlefield and unlike other evolutionary processes, the host-pathogen evolutionary warfare leaves numerous evidences in the genome which can be tracked with relative ease.

Aim:

The aim of the present study will be to mine evidences of molecular arms race between primate immune system and certain pathogenic bacterial strains.

Objective:

- Selection of the bacterial strains which have been known to evade host immune response and retain their status as potent infective agent in primates.

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- Detection of genetic loci within the bacterial strains and primate genome, as candidates for understanding arms race.
- Depicting the pattern of evolutionary arms race between the pathogens and their respective hosts.

Materials and methods:

a.) Selection of Bacterial Strains:

Pathogenic bacteria use different strategies to evade the immune system. Antigenic variation, latency, resistance to immune effector mechanisms, and suppression of the immune response all contribute to persistent and medically important infections (Janeway *et al*; 2001).

Pestilential primate bacterial strains would be selected. Different bacterial species might evolve different strategies of infection as well as immune system evasion (Hawash *et al*; 2021). While selecting bacterial species, care will be taken not to include closely related species so that novel strategies may be identified. Also, overlapping strategies may be tracked.

The other points to be taken care while selection of bacterial strains would be:

- Stable pathogenicity: A pathogen which has retained its status as a primate infective agent since a long time in the evolutionary history (Devaux *et al*; 2019).
- Non-lethal pathogens: Usually pathogens evolving lethal traits either wipes off the hosts by taking pandemic proportions or are met with an equally threatening immune response which wipes off the pathogen. This ceases the arms race and both host and pathogen lose their status (Janeway *et al*; 2001). Therefore, strains which are infective but non- lethal will be selected.
- Transmissibility and infectivity: Bacterial strains of varied infectivity and transmissibility will be selected so that the broad range of strategies evolved in

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each case can be appreciated (cressler *et al*; 2016).

Taking the above factors into consideration, databases such as 'The Virulence Factor Database' (VFDB) will be utilized for selection of primate bacterial strains (Chen *et al;* 2005).

b.) Detection of genetic loci (pathogenic and host) exhibiting arms race:

- Detection of immune system genes:

Detection of appropriate immune system genes is very important. The evolution of mammalian immune system has a strong relation with the varied infection and selection sweeps in the evolutionary history (Gerardo *et al*; 2020). For example, some infections spread by immune activation, and would not spread if it were not for the immune response. In these situations, the immune response contributes to the issue. While studying the immune system as a whole might not be within the scope of the thesis work, care will be taken to study the evolutionary dynamics of important immune system loci which have been under selection and studied for signatures of arms race.

Databases such as the 'Immune Epitope Database' will be employed for selection of appropriate immune genes (Martini *et al;* 2020).

- Detection of potent genes involved in the arms race in the pathogen:

Varied physiological adaptations are observed in prokaryotes, such as the use of different carbon sources and electron acceptors, resistance to stressors and molecular interactions with host cells (Brbic *et al;* 2016). Careful search strategies will be employed to look for genes directly involved in and evolving to evade the host immune system and cause infection. For this too, the Virulence factor data base (VFDB) will be employed (Chen *et al;* 2005).

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- Detection of fast evolving genes in both host and pathogen:

Powerful methods have been developed for detecting targets of selection in the genome without any a priori knowledge (Meyers *et al;* 2003, Nielsen; 2005, Nielsen *et al*; 2005, Tenaillon and Tiffin; 2008). Such an approach detects the most rapidly evolving genes (e.g., those subject to positive selection) in host and pathogen genomes. More recently, technological and methodological progress has allowed the detection of footprints of selection directly on genes. For instance, genes assumed to be important in host–pathogen coevolution can be amplified, sequenced and analysed by powerful methods to check whether they are indeed targets of selection, what evolutionary forces are at play (e.g., balancing selection or selective sweeps), what the strength of selection is, and whether selection has been acting in the more or less recent past (Nielsen; 2005, Tenaillon and Tiffin; 2007). Such information can provide useful insights into the processes of coevolution.

- Strategies for detecting selection:

Pathogenicity associated Islands: Several bacterial diseases have unique genetic regions on their chromosomes called pathogenicity islands (PAIs). Non-pathogenic strains of the same or very related species typically lack PAIs, which encode a variety of virulence factors. PAIs are regarded as a subclass of genomic islands that contribute to "quantum leaps" in microbial evolution (Gal-Mor *et al;* 2006). They are obtained through transduction, conjugation, and transformation, which are forms of horizontal gene transfer. Recent research reported that PAIs have not only led to the identification of many novel virulence factors used by these species during infection of their respective hosts, but also dramatically changed our way of thinking about the evolution of bacterial virulence (Schmidt *et al;* 2004). Various computational methods will be used for

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identification of PAIs such as GC content, GC skew, Codon usage, percentage of phage related genes, presence of direct repeats, insertion sequence etc (Che *et al;* 2014) and their significance in the bacterial genome will be work out.

Measuring the dN/dS ratio:

Inter-species approaches rely on the alignment of orthologous coding sequences, analyse these alignments site-by-site, and at each site determine which, among all possible substitutions, would be non-synonymous (amino acid replacing) or synonymous (non-amino acid replacing). The ratio of non-synonymous to synonymous substitutions (dN/dS) is a useful measure of the strength and mode of natural selection acting on protein-coding genes (Nielsen,2005). Measuring the dN/dS ratio (number of nonsynonymous vs synonymous substitutions over number of synonymous substitutions) provide direct way to measure the selective pressure acting on codons. In general, a dN/dS >1 indicates positive selection, <1 indicates purifying selection and a ratio at or close to 1 is neutral selection (Kryazhimskiy *et al* ;2008). A number of computer tools have come up for analysis of selection. CodeML is one such tool which utilises the PAML package (Yang 2007). CodeML or Easy CodeML will be utilised for this purpose.

c.) Depicting patterns of evolutionary arms race

Computer packages such as CodeML will be utilised for calculating and matching the pace of selection between pairs of bacteria and immune genes so that pattern of evolutionary arms race may be predicted (Shultz *et al;*2019).

Expected Results

The proposed study is expected to provide novel insights into the different strategies employed by bacteria and their primate hosts for a continued arms race.

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References

Aguileta G, Refregier G, Yockteng R, Fournier E, Giraud T. (2009) Rapidly evolving genes in pathogens: methods for detecting positive selection and examples among fungi, bacteria, viruses and protists. Infect. Genet. Evol.; 9(4):656–670.

Brbić M, Piškorec M, Vidulin V, Kriško A, Šmuc T, Supek F. (2016) The landscape of microbial phenotypic traits and associated genes. Nucleic Acids Res. Dec 1;44(21):10074-10090. doi: 10.1093/nar/gkw964.

Che, D., Hasan, M. S., & Chen, B. (2014). Identifying pathogenicity islands in bacterial pathogenomics using computational approaches. Pathogens (Basel, Switzerland), 3(1), 36–56. https://doi.org/10.3390/pathogens3010036.

Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., & Jin, Q. (2005). VFDB: a reference database for bacterial virulence factors. Nucleic acids research, 33(Database issue), D325–D328. https://doi.org/10.1093/nar/gki008.

Cressler, C. E., McLEOD, D. V., Rozins, C., VAN DEN Hoogen, J., & Day, T. (2016). The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. Parasitology, 143(7), 915–930. https://doi.org/10.1017/S003118201500092X

Daugherty MD, Malik HS. (2012) Rules of Engagement: Molecular Insights from Host-Virus Arms Races. Annu RevGenet.;46:677–700.https://doi.org/10.1146/annurev-genet-110711-155522.

Devaux, C. A., Mediannikov, O., Medkour, H., & Raoult, D. (2019). Infectious Disease Risk Across the Growing Human- Non Human Primate Interface: A Review of the Evidence. Frontiers in public health, 7, 305. https://doi.org/10.3389/fpubh.2019.00305.

Enard D, CaiL, Gwennap C, Petrov DA. (2016) Viruses are a dominant driver of protein adaptation in mammals. Elife.; 5: 56.

Gal-Mor, O., & Finlay, B. B. (2006). Pathogenicity islands: a molecular toolbox for bacterial virulence. Cellular microbiology, 8(11), 1707–1719. https://doi.org/10.1111/j.1462-5822.2006.00794.

Gerardo NM, Hoang KL, Stoy KS. (2020) Evolution of animal immunity in the light of beneficial symbioses. Philos Trans R Soc Lond B Biol Sci. Sep 28;375(1808):20190601. doi: 10.1098/rstb.2019.0601.

Haldane J. (1949) Disease and evolution. La Ricerca Scientifica Supplemento.; 1–11.

Hamilton WD, Axelrod R, Tanese R. (1990) Sexual reproduction as an adaptation to resist parasites (a review). Proc Natl Acad Sci USA.;87:3566–3573. https://doi.org/10.1073/pnas.87.9.3566.

Hawash MBF, Sanz-Remón J, Grenier JC, Kohn J, Yotova V, Johnson Z, Lanford RE, Brinkworth JF, Barreiro LB. (2021) Primate innate immune responses to bacterial and viral pathogens reveals an evolutionary trade-off between strength and specificity. Proc Natl Acad Sci U S A. Mar 30;118(13): 2015855118. doi: 10.1073/pnas.2015855118.

Hawkins JA, Kaczmarek ME, Mu ¨ller MA, Drosten C, Press WH, Sawyer SL. (2019) A meta-analysis of bat phylogenetics and positive selection based on genomes and transcriptomes from

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18 species. Proc Natl Acad Sci USA.; 116:11351-11360. https://doi.org/10.1073/pnas.1814995116.

Janeway CA Jr, Travers P, Walport M, et al. (2001) Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science. Pathogens have evolved various means of evading or subverting normal host defenses. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27176/.

Laine, A. L., Burdon, J. J., Dodds, P. N., & Thrall, P. H. (2011). Spatial variation in disease resistance: from molecules to metapopulations. The Journal of ecology, *99*(1), 96–112. https://doi.org/10.1111/j.1365-2745.2010.01738.

Martini S, Nielsen M, Peters B, Sette A. (2020) The Immune Epitope Database and Analysis Resource Program 2003-2018: reflections and outlook. Immunogenetics. 72(1-2):57-76. doi: 10.1007/s00251-019-01137-6.

Nielsen R, Bustamante C, Clark AG, Glanowski S, Sackton TB, Hubisz MJ. (2005). A scan for positively selected genes in the genomes of humans and chimpanzees. PLoSBiol.;3: e170.https://doi.org/ 10.1371/journal.pbio.0030170.

Sackton TB, Lazzaro BP, Schlenke TA, Evans JD, Hultmark D,Clark AG. (2007) Dynamic evolution of the innate immune systemin Drosophila. Nat Genet.; 39: 1461–1468. https://doi.org/10.1038/ng. 2007.

Schmidt, H., & Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. Clinical microbiology reviews, 17(1), 14–56. https://doi.org/10.1128/CMR.17.1.14-56.

Shultz, A. J., & Sackton, T. B. (2019). Immune genes are hotspots of shared positive selection across birds and mammals. eLife, 8, e41815. https://doi.org/10.7554/eLife.41815.

Van Valen L. A new evolutionary law. Evol. Theory.1973; 1: 1–30.

Vander Lee R, WielL, VanDam TJP, Huynen MA. (2017). Genome scale detection of positive selection in nine primates predicts human-virus evolutionary conflicts. Nucleic Acids Res. https://doi.org/10.1093/ nar/gkx704.

Woods RJ, Barrick JE, Cooper TF, Shrestha U, Kauth MR, Lenski RE. (2011). Second-order selection for evolvability in a large Escherichia coli population. Science.; 331(6023):1433–1436.

Yang, Z. (2007). PAML 4: a program package for phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution 24: 1586-1591.

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